1,152,679

PATENT SPECIFICATION

NO DRAWINGS

1,152,679

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COMPLETE SPECIFICATION

Pharmaceutical and Veterinary Preparations

We, COMMERCIAL SOLVENTS CORPORATION, a corporation organized and existing under the laws of the State of Maryland, United States of America, of 260 Madison Avenue, New York, State of New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is directed to pharmaceutical and veterinary compositions useful for instance, in fertility control, estrogen therapy and in increasing anabolic activity in animal feeds containing the compositions, in methods of making such animal feeds, and in methods of so treating animals by administration of such compositions and such feeds.

Sex hormones, including estrogens, progestens and androgens have found wide use as growth-promoting agents in the therapeutic treatment of humans and animals alike. Equally as common has been the use of these hormones, particularly mixtures of progestogens and estrogens, in fertility control. In each of these areas, however, the major drawback to the use of the hormones has been due to relatively severe side effects.

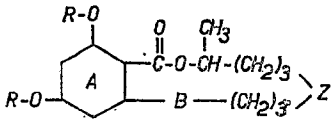
It has now been found that a pharmaceutical and veterinary composition containing a new combination of at least two active ingredients provides the desired therapeutic anabolic or anti-fertility activity with minimization of undesirable side effects and in many instances, the combinations of the invention provide compositions more active than the sum of activities of the individual active ingredients. In addition, it has been found that the new combination of at least two active ingredients can be administered separately approximately simultaneously in the same or different manner or medium to obtain the desired therapeutic, anabolic or anti-fertility activity with similar minimization of undesirable side effects.

Each of the active ingredients of the invention will be discussed below under a separate heading.

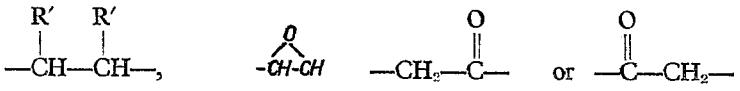
The term "lower" as used throughout the description and claims is intended to limit the radicals to having from 1 to 6 carbon atoms.

FIRST ACTIVE INGREDIENT

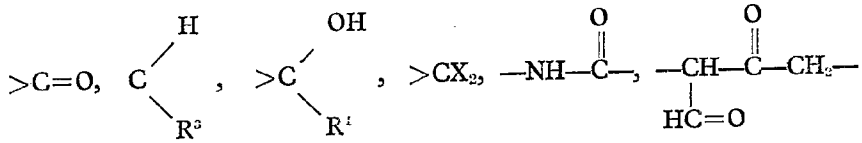
The compounds which constitute the first active ingredient of the composition of the invention can be represented by the structural formula:

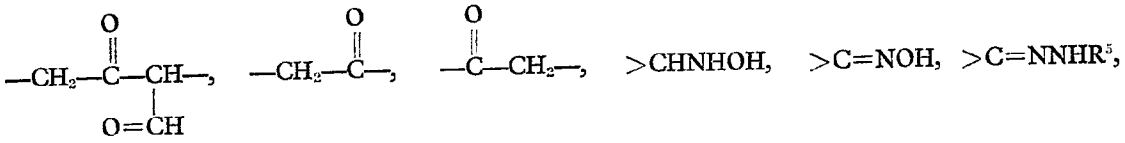


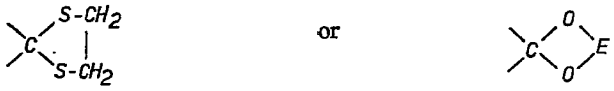
wherein R is hydrogen, lower alkyl, lower saturated acyclic acyl or benzyl; B is —CH=CH—,



wherein R′ is a hydrogen atom, fluoride, hydroxyl or —N(R²)₂ wherein R² is a hydrogen atom, an alkyl having 1 to 15 carbon atoms, monocyclic aryl or an aralkyl having 1 to 15 carbon atoms; Z is







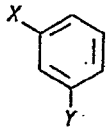
wherein R³ is a hydrogen atom, an hydroxyl group, a halogen atom, —OR⁶ or —NHR⁷, R⁶ is lower alkyl, benzyl or lower saturated acyclic acyl and R⁷ is a hydrogen atom, lower alkyl or monocyclic aryl; R⁴ is alkyl, aryl, aralkyl, nitromethyl, aminomethyl, ethynyl, cyano or —R⁹COOR⁸ wherein R⁸ is lower alkyl and R⁹ is lower alkylene; X is a halogen atom, nitromethyl cr aminomethyl; R⁵ is lower alkyl, hydrogen, carbamyl or a mono-cyclic aryl; and E is an alkylene radical having 2 or 3 carbon atoms; with the proviso that when Z is one of

$>CH_2$, $>CHNHR^7$ or $>CHNHOH$,

B is $—CH_2—CH_2—$;

and

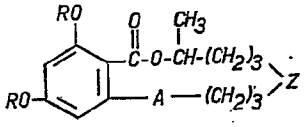
is a cyclo-hexane ring or a benzene ring of the formula



wherein X and Y are each a hydrogen, atom, nitro, amino, diazonium radical, cyano, hydroxyl, aryl, alkenyl, alkyl, acyl, alkoxy, carboxy, —SO₃H, —SO₃R¹⁰, —SO₂Cl or —SO₂NHR¹¹ wherein R¹⁰ is lower alkyl and R¹¹ is a hydrogen atom, an alkyl having from 1 to 20 carbon atoms, monocyclic aryl or a halogen atom, with the proviso that when one of X and Y is carboxy the other of X and Y is a hydrogen atom, that R is lower alkyl or benzyl when X or Y is —SO₂Cl, and that when

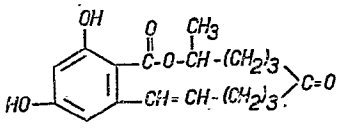
is a cyclohexane ring, B is $—CH_2—CH_2—$ and Z is $>CH_2$ or $>CHOR^6$.

Especially preferred compounds constituting the first active ingredient are represented by the structural formula:



wherein R is a hydrogen atom, lower alkyl such as methyl, ethyl or hexyl, or acyclic acyl radicals such as acetyl and valeryl; A is $—CH_2—CH_2—$ or —CH=CH— and Z is $>C=O$, $>CH_2$ or $>CHOH$.

These compounds constituting the first active ingredient include:



hereinafter referred to as the fermentation estrogenic substance (F.E.S.), and other com-

pounds produced by reduction of the ketone group to replace the oxygen of the ketone group with two hydrogen atoms, by reduction of the ketone group to add two hydrogens thereto, by saturation of the olefinic bond or by any combination of such reductions. The reduction of the ketone group to replace the oxygen can be effected by any one of several procedures including the Clemmensen reduction using zinc and hydrochloric acid, the Wolff-Kishner reduction using hydrazine and alkali, e.g., NaOH, and the formation of the dithioacetal with ethylene dithiol or ethylmercaptan and catalytic desulfurization with Raney nickel catalyst containing adsorbed hydrogen.

The addition of the two hydrogen atoms to the ketone group and saturation of the olefinic bond can be accomplished by conventional reduction procedures, for instance, in the presence of Raney nickel catalyst. The reduction is preferably carried out with the F.E.S. suspended or dissolved in a suitable solvent, e.g., an alcohol, preferably a lower alkanol such as methanol or ethanol. In general, the reduction can be accomplished at ambient temperatures and ambient pressures. Preferable temperatures are from 15° to 40°C., and preferable pressures are of from 1 to 100 atmospheres. In general, from 0.1 to 5 grams of catalyst are used per gram of F.E.S.

In producing compounds of the invention where A is $-CH_2-CH_2-$ the olefinic bond of F.E.S. can be reduced, for example, by hydrogenation in the presence of a Group VIII metal, particularly platinum or palladium catalyst on a suitable carrier, e.g., charcoal. Generally, the catalyst contains from 0.01 to 10% of the catalytic metal. The catalyst is used in a ratio of generally from 0.02 to 2 grams of catalyst, preferably 0.1 to 0.5 gram, and particularly 0.2 gram of catalyst per gram of F.E.S. The reduction may be carried out while the F.E.S. is dissolved in a suitable solvent, e.g., an alcohol, especially a lower alkanol such as 2-propanol, methanol, ethanol or an acid, e.g. acetic acid, at ambient temperatures, e.g. from 15° to 40° C., and ambient pressures, since only the presence of hydrogen is required. It is preferred, however, to utilize an elevated pressure, e.g. from 1 to 50 atmospheres of hydrogen.

In producing compounds of the present invention where R is alkyl, conventional alkylation procedures can be used to replace the H atom of one or both of the OH groups on the benzene ring of F.E.S. with an alkyl group. Alkylated dihydro F.E.S. compounds can be produced, for example, by first alkylating F.E.S. and then reducing it as set forth *supra*, or by first reducing it and then alkylating it. The alkylation can be by reaction with the corresponding dialkyl sulfates, e.g., dimethyl sulfate, or diethyl sulfate, to produce the dialkyl F.E.S. or a monoalkyl F.E.S. with the alkyl group replacing the hydrogen of the hydroxyl group on the benzene ring ortho to the ester group. Furthermore, a monomethyl F.E.S. compound with the methyl group replacing the hydrogen of the hydroxyl group para to the ester group can be selectively produced using diazomethane.

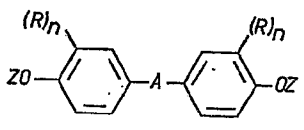
In producing compounds of the present invention where R is acyl, conventional acylation procedures can be used to replace the hydrogen atoms of one or both of the hydroxyl radicals on the benzene ring of F.E.S. with an acyl radical. Acylated F.E.S. compounds can be produced, for example, by reaction with the corresponding acid anhydride, e.g. acetic anhydride or propionic anhydride, catalyzed with, for example, sodium acetate or pyridine. Ambient conditions can be used although it is preferred to keep the reaction mixture cold. When producing compounds having one R as alkyl and the other acyl, it is advantageous to alkylate before acylating.

The fermentation estrogenic substance (F.E.S.) is so named since a convenient method for producing it is by cultivating, on a suitable nutrient medium, the organism *Gibberella zeae* (Gordon) on deposit at the Northern Utilization Research and Development Division of the United States Department of Agriculture under the number NRRL-2830.

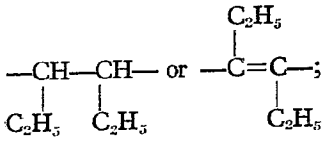
Specific examples of the preparation of F.E.S. and other compounds of the invention are given below and disclosed in more detail in United Kindom Patent Nos. 1, 107, 735; 1,105, 894; 1,107, 739; 1,107, 737; 1,107, 738; 1,107, 732; 1,105, 895; 1,107, 744; 1,107, 742; 1,107, 736; 1,107, 734; 1,107, 745; 1,107, 741; 1,107, 743; 1,107, 740; and in United States patent Nos: 3,196, 019; 3,373, 024; 3,373, 028, 3,373, 027; 3,373, 039; 3,373, 036; 3,373, 030; 3,373, 029; 3,373, 026; 3,373, 032; 3,373, 033; 3,373, 038; 3,373, 034; 3,373, 037; 3,373, 035; and Canadian Patent No. 784,752.

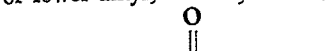
Second Active Ingredient

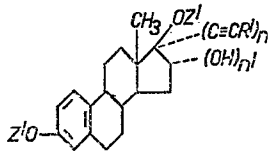
The substance which constitute the second active ingredient of the compositions of the invention are known estrogens and progestogens and include: 1) benzestrol; 2) dienestrol; 3) estrone; 4) 3-(6-methoxy-2-naphthyl)-2, 2 - dimethylpentanoic acid; 5) diethylstilbestrol and its derivatives as represented by the structure:



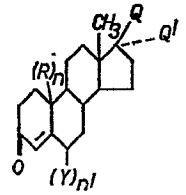
wherein A is



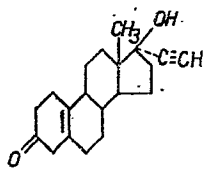
R is H or lower alkyl; Z is H, $-PO_3$, $-SO_3$, $-R'$ or  wherein R is an aliphatic hydrocarbon radical of 1 to 20 carbon atoms; n is 0 to 1 and when 0, R is H; 6) estradiol and its derivatives as represented by the structure:



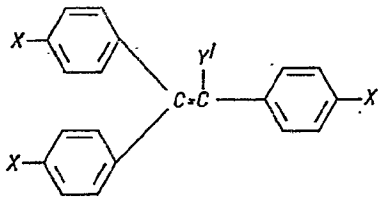
wherein Z' is H, benzoyl, —R,  where R is lower alkyl, (including cycloalkyl); R' is H or lower alkyl; n and n' are 0 or 1; and when 0, C≡CR' is H; 7) progestogens having the structure:



or



wherein R is H or lower alkyl; Q is —OH,  where R is lower alkyl; Q' is H, acetoxy —C≡CH, —C≡CR or where R is lower alkyl; Y is lower alkyl or halogen, preferably a halogen having an atomic number of 17—53 including chlorine, bromine or iodine; and n and n' are 0 or 1 and when n is 0, R is H; and when n' is 0, Y is H; 8) a tris-phenyl ethylene having the structure:



wherein X is —OH, lower alkoxy and acyloxy; and Y' is a halogen atom, preferably having an atomic number of 17—53 or H.

Illustrative of derivatives of diethylstilbestrol that may be used, for instance, are diethylstilbestrol dipropionate, diethylstilbestrol monomethyl ether, diethylstilbestrol dimethyl ether, diethylstilbestrol dipalmitate, diethylstilbestrol diphosphate, diethylstilbestrol disulfate, hexestrol and promethestrol dipropionate. Exemplary of suitable estradiol derivatives under the above structure are estradiol benzoate, estradiol cyclopentyl propionate, estradioldipropionate, estriol, ethinyl estradiol, ethinyl estradiol - 3 - methyl ether. Suitable progestogens include, for instance, progesterone, dimethisterone, 17α-ethinyl-17β-hydroxy-5 (10) estren-3-one (norethynodrel), norethindrone, chlormadinone acetate and medroxy-progestrone acetate. Examples of suitable tris-phenyl ethylene compounds are tris (p-methoxy phenyl) ethylene, tris-(p-hydroxyphenyl) ethylene and tris-(p-methoxy phenyl)chloro-ethylene.

The pharmaceutical compositions of the invention can be prepared by mixing the active ingredients with non-toxic, pharmaceutically-acceptable carriers, which can be inert diluents or solid carriers, and forming the resulting mixture into suitable dosage unit forms. The compositions can be administered to the subject by any suitable method including oral and parenteral administration. Forms suitable for oral administration include, for example, pressed or coated tablets, capsules or pills, syrups, solutions or suspensions in water or non-toxic organic solvent media such as propylene glycol and glycerol formal, and dispersible powders. Compositions suitable for parenteral administration are the known pharmaceutical forms for such administration, for example, sterile aqueous solutions or suspensions in oily media. The sterile aqueous solutions can be formulated in the presence of parenterally acceptable buffers, e.g. sodium citrate, citric acid and/or preservatives such as phenol and methyl and propyl esters of p-hydroxy benzoic acid. A preferred oily media for preparation of the sterile solution is peanut oil or peanut oil and benzyl alcohol.

The pharmaceutical and veterinary com-

positions may also include adjuvants known in the art as desirable or useful as, for example, wetting agents, dispersing agents, suspending agents, lubricating agents, sweetening agents, coloring agents and flavoring agents.

Illustrative of oral compositions are tablets wherein the active ingredients are mixed with inert fillers, e.g. dicalcium phosphate, terra alba or lactose in the presence of disintegrating agents as, for example, maize or starch and in the presence of lubricating agents such as magnesium stearate. Examples of suitable aqueous solutions for oral use are those formulated by incorporating the active ingredients in inert pharmaceutically-acceptable liquid solvent media which can contain, if desired, pharmaceutically-acceptable thickening agents such as sodium carboxymethylcellulose and/or pharmaceutically-acceptable sweetening and flavoring agents.

The actual total amounts of the active ingredients in the pharmaceutical or veterinary compositions may vary depending upon the particular activity desired or disorder treated but in all cases the total amount is that effective to produce the desired therapeutic, anabolic or anti-fertility effect. In general the amount is from 0.2 to 500 mg., preferably 1.0 to 100 mg. per dosage unit. The proportions of first active ingredient to second active ingredient may vary widely and ordinarily are in a weight ratio of from 300:1 to 1:300.

The particular proportions employed in a given instance may be dependent on specific ingredients selected and the result desired.

The following Examples illustrate preparation of the first active ingredient employed in the compositions of the invention. The first Example illustrates preparation of a suitable innoculum containing the organism *Gibberella zeae* (Gordon) NRRL-2830.

Example I

A spore sand culture containing *Gibberella zeae* (Gordon) NRRL-2830 was asceptically placed in a sterile tube containing 15 milliliters of Czapek's-Dox solution and a small amount of agar. This medium was then incubated for 168 hours at 25° C. At the end of the incubation period, the medium was washed with 5 milliliters of sterile deionized water and transferred to a sterile tube containing 45 milliliters of Czapek's-Dox solution. The contents of the tube were then incubated for 96 hours at 25°C. after which the material was available for use in innoculation of a fermentation medium.

The following Example illustrates the fermentation of the organism *Gibberella zeae* (Gordon) NRRL-2830 to produce F.E.S.

Example II

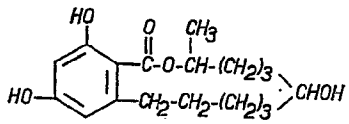
To a 2 liter flask were added 300 grams of finely divided corn. The flask and its contents were then sterilized and after sterilization, 150 milliliters of sterile deionized water were added. To the mixture in the flask were then added 45 milliliters of the inoculum prepared by the process of Example I and the material was thoroughly mixed. The mixed material was then incubated for 20 days at 25°C. in a dark room in a water-saturated atmosphere.

The following Example illustrates the recovery of the F.E.S. from the fermentation medium.

Example III

A 300 gram portion of fermented material produced by the method of Example II was placed in 500 milliliters of deionized water and slurried. The slurry was then heated for 15 minutes at 75°C., 300 grams of filter aid were then added and the material was filtered. The solid filtered material containing the F.E.S. was then air dried, and 333 grams of the dried cake were then extracted with 500 milliliters of ethanol. This procedure was repeated three more times. The ethanol extract was evaporated to dryness under vacuum to give 6.84 grams of solid material. This solid material was then dissolved in 20 milliliters of chloroform and extracted with 30 milliliters of an aqueous solution containing 5% by weight of sodium carbonate having an adjusted pH of 11.2. The extraction process was repeated seven more times. The pH of the sodium-carbonate extract was then adjusted to 6.2 with hydrochloric acid, to yield F.E.S. containing precipitate. The precipitate and the aqueous sodium carbonate extract were then each in turn extracted with 75 milliliters of ethyl ether. This procedure was repeated three more times to yield a light yellow ethereal solution, which was then evaporated to yield 116 milligrams of solid F.E.S. This material was then subjected to multiple transfer countercurrent distribution using 100 tubes and a solvent system consisting of two parts chloroform and two parts carbon tetrachloride as the lower phase and four parts methanol and one part water as the upper phase, all parts by volume. The solid material obtained from the multiple transfer countercurrent distribution was F.E.S.

The following Examples, Examples IV to VI, illustrate the reduction of F.E.S. to produce tetrahydro F.E.S. having the formula:



Example IV

Tetrahydro F.E.S. was produced by dissolving 0.5 gram F.E.S. in 200 milliliters of ethanol. The F.E.S. was reduced by contacting the solution with hydrogen for 3 hours at

30°C. with 1000 psi using 2 grams of Raney nickel as a catalyst. After filtering and concentrating the reaction mixture, the product was washed with 2 to 3 milliliters of 2-nitropropane and crystallized. It was found to have a melting point of from 143° to 160°C.

EXAMPLE V

The reduction of F.E.S. was conducted in methanol at 30°C. and 1000 psi hydrogen pressure for 5 hours using Raney nickel catalyst to provide a product melting, after several crystallizations from 2-nitropropane and nitromethane, at from 141° to 143°C. and analyzing:

Calc. ($C_{18}H_{26}O_5$)		Found
% C	67.1	67.2
% H	8.14	8.28

EXAMPLE VI

The reduction of 1 gram of F.E.S. was conducted in 150 cc. of ethanol at room temperature and 50 psi of hydrogen for 4 hours in the presence of a small amount of Raney nickel (about 1 cc. of a thick suspension in water). The product was concentrated, treated with 5 milliliters of isopropyl alcohol, cooled and filtered. The filtrate was mixed with 5 milliliters of water, left standing overnight, cooled and filtered to provide 0.65 gram of product having a melting point of from 147° to 157°C. This product was recrystallized from isopropyl alcohol-water mixtures two times to provide 0.18 gram of a product having a melting point of from 187° to 180°C. A product having a melting point of from 146° to 148°C., and weighing 0.22 gram was also recovered from the filtrate after the first recrystallization of the product weighing 0.65 gram. The reduction of the ketone group introduces an asymmetric carbon atom and makes diastereoisomers possible. The optical activities of the two products were (1) for the product with a melting point of from 178° to 180°C., $[\alpha]_D^{25}=+46°$ e.g. and (2) for the product with a melting point of from 146° to 148°C., $[\alpha]_D^{25}=+39°$ where $[\alpha']=\frac{\alpha.100}{c.l}$

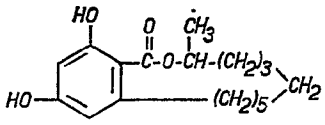
c=1% in. methanol and l=2 dcm.

The following Example illustrates the preparation of deoxy tetrahydro F.E.S.

EXAMPLE VII

Two 10 gram portions of F.E.S. each in 200 milliliters acetic acid, were catalytically reduced at room temperature in the presence of 1.2 grams of PdO catalyst at a hydrogen pressure of 45 psi. The combined reduction mixtures were heated to boiling, filtered, and the filter cake was washed with 50 milliliters of hot acetic acid. The cooled filtrate was added, with stirring, to 2 liters of water. The mixture was stirred for 15 minutes and the white solid was collected by filtration, washed and dried in a vacuum desiccator to yield 19.1 grams of dihydro F.E.S. having a melting point of from 191° to 193°C.

The dihydro F.E.S. (1 gram) is added slowly with cooling (ice-bath), to a mixture of 5 cc. of ethylene dithiol .25 gram of freshly fused zinc chloride and 2 grams of anhydrous sodium sulfate, contained in a microflask. The mixture is maintained at 5°C. for 20 hours and then at room temperature for 4 hours, whereupon it is poured into 50 cc. of ice and the precipitate is collected and subjected to hydrogenolysis. To the reaction product is added 100 cc. of 90% ethanol and 15 grams of Raney nickel catalyst and the mixture is refluxed until the reaction is complete. The nickel is removed by centrifugation and is washed several times with hot ethanol by centrifugation followed by decantation, and the centrifugates are combined. The mixture is evaporated to dryness and the residue is suitably recrystallized to yield a compound having the formula:



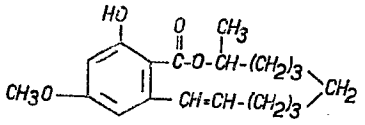
EXAMPLE VIII

Nitrosomethylurea in an amount of 1.2 grams was slowly added to a cold mixture of 3.6 milliliters of 50% potassium hydroxide and 17 milliliters of ether. After a few minutes the yellow ether layer of the mixture was decanted, dried over potassium hydroxide, and then added to a solution of 0.30 gram F.E.S. in 17 milliliters of ether. The resulting yellow mixture was left overnight in a loosely stoppered flask and then ether and diazomethane were evaporated using a steam bath. The remaining gummy residue was crystallized by adding 3 milliliters of water, heating to 60°C., and adding ethanol almost to solution. On cooling, crystals formed yielding 0.137 gram of a product having a melting point of

from 111° to 116°C. which was recrystallized in the same way to yield 0.082 gram of monomethyl F.E.S. having a melting point of from 120° to 122°C. and analyzing:

Calc. ($C_{19}H_{24}O_5$)		Found
% C	68.7	68.3
% H	7.28	7.38
% OCH_3	9.34	9.17

The *p* methyl F.E.S. is substituted for the dihydro F.E.S. in following essentially the same procedure used in Example VII to produce a compound having the formula:



The following Example illustrates the production of dimethyl F.E.S. and monomethyl F.E.S. derivatives, the monomethyl F.E.S. derivative having the hydrogen in the hydroxyl group ortho to the ester group replaced with a methyl group.

EXAMPLE IX

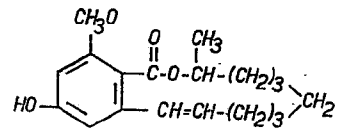
Dimethyl sulfate (5 milliliters) was added to a solution of 2.24 grams of F.E.S. in 80 milliliters 10% NaOH and 20 milliliters water. The mixture was stirred for one-half hour at from 18° to 20°C. (cooling bath) and an additional 5 milliliters of dimethyl sulfate was added. After an additional 70 minutes of stirring at from 20° to 26°C., the solid precipitate, Solid A, was collected by filtration, washed with water and dried in a vacuum desiccator. The filtrate from Solid A was acidified with 25 milliliters 12 NH_2SO_4 to yield a second precipitate, Solid B, which was collected, washed with water, and dried.

Solid A (0.79 gram having a melting point of 114°—118°C) was recrystallized from a mixture of 10 milliliters water and 15 milliliters ethanol to yield 0.66 gram of dimethyl F.E.S. having a melting point of from 108° to 110°C.

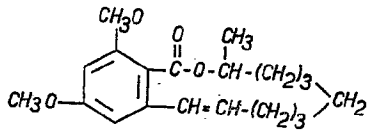
Solid B (1.39 grams having a melting point of from 152° to 162°C) was recrystallized twice from a mixture of water and alcohol to yield 0.8 gram of monomethyl F.E.S. having a melting point of from 169° to 174°C. and the following analysis of recrystallized Solid B (monomethyl F.E.S.) was obtained:

Calc. ($C_{19}H_{24}O_5$)		Found
% C	68.65	67.97
% H	7.28	7.16
% OMe	9.34	9.28

Each of the *o* methyl F.E.S. and the dimethyl F.E.S. is substituted for the dihydro F.E.S. in the procedure of Example VII to produce the respective compounds:



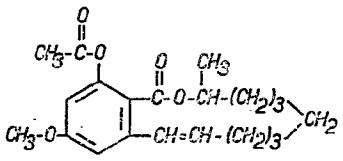
and



The following Example illustrate the production of an acylated monomethyl F.E.S. derivative.

EXAMPLE X

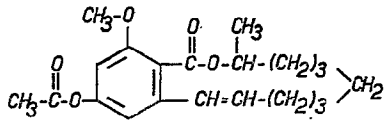
To a solution of 368 milligrams of *p* methyl F.E.S. in 8 milliliters pyridine is added 5 milliliters acetic anhydride and the mixture is held at room temperature for 16 hours. Twenty-five milliliters of water are then added. The mixture is stored in a refrigerator for 2 hours. The solid precipitate is collected by filtration, washed with water and dried in a vacuum desiccator to recover a compound which is substituted for the dihydro F.E.S. in the procedure of Example VII to produce a compound of the formula:



which is recovered.

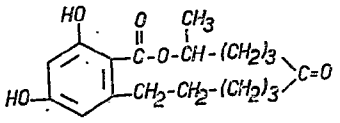
EXAMPLE XI

The compound:



is produced by the method of Example VII by substituting an acylated *o*-methyl F.E.S. derivative for the dihydro F.E.S. used in Example VII.

The following Example illustrate the reduction of F.E.S. to produce dihydro F.E.S. having the structure:



EXAMPLE XII

Two 10-gram portions of F.E.S. each in 200 milliliters acetic acid were catalytically reduced at room temperature in the presence of 1.2 grams of PdO catalyst at a hydrogen pressure of 45 psi. The combined reduction mixtures were heated to boiling, filtered, and the filter cake was washed with 50 milliliters of hot acetic acid. The cooled filtrate was added, with stirring, to 2 liters of water. The mixture was stirred for 15 minutes and the white solid was collected by filtration, washed and dried in a vacuum desiccator to yield 19.1 grams of dihydro F.E.S. having a melting point of from 191° to 193°C.

The production of dimethyl dihydro F.E.S. is illustrated by the following Example.

EXAMPLE XIII

Dihydro F.E.S. (556 milligrams) was dissolved in 25 milliliters 10% NaOH and 10 milliliters water and the solution was stirred. To the stirred solution was added three, two-milliliter portions of dimethyl sulfate at half-hour intervals followed by stirring for an additional hour. The mixture was acidic and it was made alkaline by the addition of 10 milliliters 10% NaOH and the alkaline mixture was stirred one-half hour. The solid formed was collected by filtration, washed with water and dried in a vacuum desiccator. The product weighed 526 milligrams and melted at from 115° to 117°C. Recrystallization from a mixture to 10 milliliters of water and 25 milliliters of ethanol provided 371 milligrams of material having a melting point of from 124° to 125.5°C. It was analyzed with the following results:

Calc. ($C_{20}H_{28}O_5$)		Found
% C	68.95	69.02
% H	8.10	8.12
% CH_3O	17.81	17.81

The following Example illustrates the production of monomethyl and dimethyl dihydro F.E.S., the monomethyl dihydro F.E.S. having a methyl group which replaced the hydrogen of the hydroxyl group on the benzene ring ortho to the ester group.

EXAMPLE XIV

The olefinic bond of each of the dimethyl F.E.S. and monoethyl F.E.S. produced as in Example IX is reduced using 50 psi of hydrogen and a small amount of 5% Pd on charcoal catalyst and conducting the reduction for 3 hours.

EXAMPLE XV

The olefinic bond of -monomethyl F.E.S. produced as in Example VIII is reduced according to the procedure of Example IX.

The following Examples illustrate pharmaceutical preparation of the present invention.

EXAMPLE XVI

A pharmaceutical preparation is prepared by admixing 4.1 grams of F.E.S. with 0.082 grams of diethylstilbestrol and triturating the mixture with 60 grams of lactose to form an homogeneous powder. To the powder is added 20 grams of silicic acid with hydrolyzed starch and water and the mixture is stirred until a homogeneous paste is formed. The paste is then dried and tableted with 2 grams magnesium stearate to form tablets containing approximately 5.1 mg. of active ingredients, i.e. 0.1 mg diethylstilbestrol and 5 mg. of F.E.S.

EXAMPLE XVII

Pharmaceutical preparation of aqueous suspension for oral administration:

Recipe for 1000 ml. of suspension

Diethylstilbestrol	0.5 g.
F.E.S.	4.5 g.
Sucrose	400.0 g.
Powdered traggacanth	7.5 g.
Flavoring essential oil	0.2 ml.
Methyl *p*-hydroxybenzoate	2.0 g.
Propyl *p*-hydroxybenzoate	0.5 g.
Glycerol	150.0 ml.
Citric Acid	2.0 g.
Benzoic Acid	1.0 g.
Distilled water	(to complete 1000. ml.)

The glycerol, benzoic acid, methyl and propyl benzoic acids, tragacanth gum, flavoring oil and active ingredients are mixed into a homogeneous mass. An aqueous solution of the citric acid is then added with slurrying and finally the sucrose is added. Slurrying is continued until an homogeneous suspension is obtained to which is added the balance of the water.

EXAMPLE XVIII

Similar tablets can be prepared by the procedure of Example XVI which contain 0.5 mg. of norethindrone, 0.8 mg. mestranol (ethinyl estradiol-3-methyl ether) and 1.5 mg. of F.E.S.

EXAMPLE XIX

4.75 mg. of F.E.S., 0.25 mg. of estrone of methyl-*p*-methoxybenzoate, 0.5 mg. of sodium citrate and 0.2 mg. of citric acid are added to 1 ml. of water. The pH of the solution is adjusted to 5 with HCl. Filtration and heat sterilization results in an aqueous solution suitable for parenteral injection.

Suitable pharmaceutical compositions can be prepared by substituting the following active ingredients in Examples XVII—XVIII for the non-steroid compound and hormone employed. therein.

Example	Non-Steroid Compound	Hormone
XX	HMTHFES(1)	benzestrol
XXI	LMTHFES(2)	(*p*-methoxy phenyl) chloroethylene
XXII	Deoxy F.E.S.(3)	dienestrol
XXIII	Dimethyl FES(4)	diethylstilbestrol dipropionate
XXIV	4-Methyl FES(5)	estradiol
XXV	2-Methyl FES(6)	estradiol benzoate
XXVI	Dihydro FES(7)	estradiol cyclopentyl propionate
XXVII	Acetyl monomethyl FES(8)	estradiol dipropionate
XXVIII	Dimethyl dihydro FES(9)	estriol
XXIX	HMTHFES(1)	estrone
XXX	Deoxy FES(3)	ethinyl estradiol
XXXI	Dimethyl FES(4)	3-(6-methoxy-2-naphthyl) 2,2-dimethylpentanoic acid
XXXII	FES	17α-ethinyl-17β hydroxy-5(10)-estren-3-one
XXXIII	LMTHFES(2)	*m,m'* dimethyl-dihydro-diethyl-stilbestrol-dipropionate
XXXIV	FES	dimethisterone
XXXV	Deoxy FES(3)	norethinodrel
XXXVI	4-Methyl FES(5)	norethindrone
XXXVII	Acetyl monomethyl FES(8)	chlormadinone acetate
XXXVIII	FES	medroxyprogesterone acetate
XXXIX	HMTHFES(1)	ethinyl estradiol-3-methyl ether
XL	FES	progesterone

Footnotes to Table

(1) High melting tetra hydro Fes prepared as in Example VI

(2) Low melting tetra hydro FES prepared as in Example VI.

(3) Prepared as in Example VII

(4) Prepared as in Example IX

(5) Prepared as in Example IX

(6) Prepared as in Example IX

(7) Prepared as in Example XII

(8) Prepared as in Example X

(9) Prepared as in Example XIII

The following Example demonstrates the unusual growth promoting activity provided by the compositions of the present invention.

EXAMPLE XLI

Nine lots of 12 lambs each were employed in the test with one lot serving as the control. The lambs were weighed and pellets of either tetrahydro F.E.S. or diethylstilbestrol or a mixture of tetrahydro F.E.S. and diethylstilbestrol were implanted under the skin of one ear of each lamb treated. Each lot of lambs was fed the identical ration for a period of 42 days. The results of the test are summarized in the following Table.

Lot	Number and Treatment(1)	Average Weight, lb.			Av. Daily Gain, lb.			Feed Conversion lbs./100 lb./wt. gain		
		Initial	21 Days	42 Days	0—21 Days	21—42 Days	0—42 Days	0—21 Days	21—42 Days	0—42 Days
1	Control	74.0	85.5	94.8	0.55	0.44	0.50	670	904	774
2	6 mg DES	74.4	90.9(2)	101.9	0.76	0.47	0.62	480	920	638
3	3 mg tetrahydro FES(3)	74.3	91.7	101.0	0.82	0.45	0.64	460	941	628
4	6 mg tetrahydro FES	74.3	88.8	102.0	0.69	0.63	0.66	531	668	597
5	9 mg tetrahydro FES	74.3	91.0	99.5	0.79	0.41	0.60	472	1013	657
6	12 mg tetrahydro FES	74.2	91.0	102.0	0.80	0.52	0.66	468	803	599
7	18 mg tetrahydro FES	74.1	89.5	101.1	0.74	0.55	0.64	490	757	604
8	6 mg tetrahydro FES + 6 mg DES	74.0	91.0	104.0	0.81	0.62	0.72	442	684	547
9	12 mg DES	73.9	89.5	99.2	0.74	0.46	0.60	472	883	629

(1) Twelve lambs per treatment.

(2) One lamb died on 15th day (pneumonia); second lamb removed on 21st day because it did not gain weight corresponding to the weight gain of other animals.

(3) 3 mg tetrahydro FES administered initially, additional individual 3 mg administered on 21st day.

The data show that the combination of tetrahydro F.E.S. and diethylstilbestrol effects better growth promotion and feed conversion than either diethylstilbestrol alone or tetrahydro F.E.S. alone.

EXAMPLE XLII

Four lots of 27 feeder lambs each were employed in the test with one lot serving as the control. The lambs were weighed and in one lot, pellets of tetrahydro F.E.S. were implanted subcutaneously in one ear of each lamb, in a second lot pellets of diethylstilbestrol were implanted subcutaneously in one ear of each lamb, and in the third lot, a pellet of tetrahydro F.E.S.* was implanted subcutaneously in one ear of each lamb and a pellet of diethylstilbestrol was implanted subcutaneously in the other ear of each lamb. Each lot of lambs was fed the identical complete pelleted ration for a period of 42 days. The results of the tests are summarized below:

Treatment Groups	Av. Daily Gain	Feed Conversion Lbs/100 lb. Gain
Control	0.50 lb.	774
12 mg. Implant Tetrahydro FES	0.66	599
12 mg. Implant DES	0.60	629
6 mg. Tetrahydro FES + 6 mg. DES Implant	0.72	547

NOTE: *The tetrahydro FES utilized in this and the following examples was HMTHFES.

EXAMPLE XLIII

Four lots of 45 feeder lambs each were employed in the test with one lot serving as the control. The lambs were weighed and in one lot, pellets of tetrahydro F.E.S. were implanted subcutaneously in one ear of each lamb, in the second and third lots, a pellet of tetrahydro F.E.S. was implanted subcutaneously in one ear of each lamb in both lots and a pellet of "Synovex" (Trade Mark) L* or a pellet of diethylstilbestrol respectively was implanted subcutaneously in the other ear of each lamb. Each lot of lambs was fed the identical complete pelleted ration for a period of 42 days. The results of the tests are summarized below:

Treatment Groups	Av. Daily Gain	Feed Conversion Lbs/100 lb. Gain
Control	0.43 lb.	862
12 mg. Implant Tetrahydro FES	0.48	766
6 mg. Tetrahydro FES + Synovex L	0.52	704
6 mg. Tetrahydro FES + 6 mg. DES	0.52	740

NOTE: *"Synovex" L is progesterone + estradiol benzoate.

EXAMPLE XLIV

Six lots of 27 feeder lambs each were employed in the test with one lot serving as the control. The lambs were weighed and in one lot, pellets of tetrahydro F.E.S. were implanted subcutaneously in one ear of each lamb, in a second lot pellets of diethylstilbestrol were implanted subcutaneously in one ear of each lamb, and in the third lot, a pellet of "Synovex" L was implanted in one ear of each lamb. In the two other lots a pellet of tetrahydro F.E.S. was implanted subcutaneously in one ear of each lamb and a pellet of diethylstilbestrol or Synovex L was implanted subcutaneously in the other ear of each lamb. Each lot of lambs was fed the identical complete pelleted ration for a period of 47 days. The results of the tests are summarized below:

Treatment Groups	Av. Daily Gain	Feed Conversion Lbs/lb. Gain
Control	0.43	8.49
12 mg. DES Implant	0.50	6.28
12 mg. P—1496 Implant	0.50	7.00
27.5 mg. Synovex Implant	0.51	6.91
6 mg. P—1496 + 6 mg. DES Implants	0.55	6.32
6 mg. P—1496 + 13.75 mg. Synovex Implants	0.48	7.08

In addition to the above animal feed examples wherein one of the two active ingredients is administered subcutaneously in separate parts of the animal body, as will be appreciated by those skilled in the area, one active ingredient can be administered subcutaneously while the other is administered orally in tablet or normal feed form, one can be administered by implant and the other by injection, one can be administered by injection and the other orally, and the like, so long as both active ingredients are administered to the animal approximately simultaneously.

Typical rations for cattle, swine, chickens, and lambs in percentages by weight are as follows:

CATTLE

Rolled Barley	40 — 43%
Molassed Dried Beef Pulp	35.5 — 37.0%
Alfalfa Pellets	8.0%
Tallow	2.5 — 3.0%
Calcium Carbonate	.35%
Urea	.30%
Phosphorus Source	.40%
NaCl	.50%
Molasses	10.00%
Trace Mineral	0.5%
Vitamin A	2—4 MMIV/Ton

SWINE

Ground Yellow Corn	77%	86—1/2%
Soybean Meal (44% protein)	16%	6—1/2%
Meat and Bone Scraps (50% protein)	2—1/2%	2—1/2%
Dehydrated Alfalfa Meal (17%)	2—1/2%	2—1/2%
Steamed Bone Meal	1/2%	1%
Ground Limestone	1/2%	.3%
Iodized Salt	1/2%	.5%
Vitamin, Antibiotic and Trace Mineral Premix	1/2%	.5%

BROILERS

	Finisher	Starter
Ground Yellow Corn	1200 lbs	1000 lbs
Soybean Meal (44% Protein)	500 lbs	700 lbs
Fish Meal (60% Protein)	80 lbs	100 lbs
Alfalfa Meal	50 lbs	50 lbs
Meat and Bone Scraps	30 lbs	0 lbs
Animal Fat	80 lbs	80 lbs
Dicalcium Phosphate	40 lbs	35 lbs
Iodized Salt	10 lbs	10 lbs
Limestone	—	15 lbs
Premix Vitamins Trace Minerals and Antibiotics	10 lbs	10 lbs

SHEEP FEED

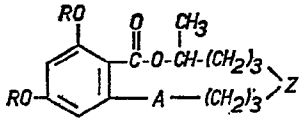
Finely Ground Corn Cobs	640 lbs
Ground Corn	600 lbs
Dehydrated Alfalfa Meal	310 lbs
Dried Molasses (85% Protein)	120 lbs
Soybean Meal (44% Protein)	300 lbs
Dicalcium Phosphate	14 lbs
Trace Mineral Salt	20 lbs
Premix Vatimin, Mineral and Antibiotic	19 lbs

In a particular aspect, the present invention provides an animal feed composition, which comprises the compositions of the present invention in admixture with a nutrient diluent. In a further aspect there is provided a method of making an animal feed composition which comprises admixing a composition of the present invention with an edible nutrient carrier.

WHAT WE CLAIM IS:—

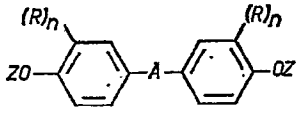
1. A pharmaceutical or veterinary composition which comprises as active ingredients in admixture with a pharmaceutically-acceptable inert carrier,

A. a compound having the structure:

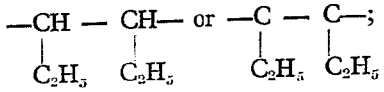


wherein R is a hydrogen atom, lower alkyl or acyclic acyl; A is $-CH_2-CH_2-$ or $-CH=CH-$ and Z is $>C=O$, $>CH_2$ or $>CHOH$ and

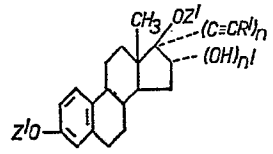
B. benzestrol, dienestrol, estrone, diethylstilbestrol or a derivative thereof as represented by the structure:



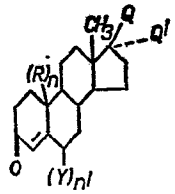
wherein A is

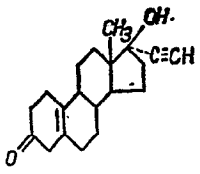


R is H or lower alkyl; Z is H, $-PO_3$, $-SO_3$, $-R'$ or $-\overset{O}{\overset{\|}{C}}R'$, where R' is an aliphatic hydrocarbon radical having from 1 to 20 carbon atoms; n is 0 or 1 and when 0, R is H; or estradiol or a derivative thereof as represented by the structure:



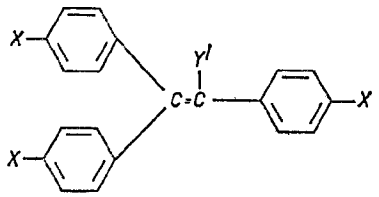
wherein Z' is H, benzoyl, R or $-\overset{O}{\overset{\|}{C}}H$, where R is lower alkyl; R' is H or lower alkyl; n and n' are 0 or 1, and when 0, $C\equiv CR'$ is H; or a progestogen having the structure:





wherein R is H or lower alkyl; Q is —OH, or

$-\overset{O}{\overset{\|}{C}}R$ and R is a lower alkyl; Q' is H, acetoxy, $-C\equiv CH$, $-C\equiv C-R$ or $-\overset{O}{\overset{\|}{C}}R$ wherein R is lower alkyl; Y is lower alkyl or a halogen atom and n and n' are 0 or 1 and when n is 0, R is H and when n' is 0, Y is H; or a tris-phenyl ethylene having the structure:



wherein X is OH, lower alkoxy or acyloxy and Y' is a halogen atom or a hydrogen atom, the weight ratio of active ingredient A to active ingredient B being from 300 to 1:1 to 300.

2. A composition as claimed in claim 1, wherein the compound B is benzestrol.

3. A composition as claimed in claim 1 wherein the compound B is tris (p-methoxyphenyl)chloroethylene.

4. A composition as claimed in claim 1, wherein the compound B is dienestrol.

5. A composition as claimed in claim 1 wherein the compound B is diethylstilbestrol dipropionate.

6. A composition as claimed in claim 1, wherein the compound B is estradiol.

7. A composition as claimed in claim 1, wherein the compound B is estradiol benzoate.

8. A composition as claimed in claim 1, wherein the compound B is estradiol cyclopentyl propionate.

9. A composition as claimed in claim 1, wherein the compound B is progesterone.

10. A composition as claimed in claim 1, wherein the compound B is estradiol dipropionate.

11. A composition as claimed in claim 1, wherein the compound B is estriol.

12. A composition as claimed in claim 1, wherein the compound B is estrone.

13. A composition as claimed in claim 1, wherein the compound B is ethinyl estradiol.

14. A composition as claimed in claim 1, wherein the compound B is 3-(6-methoxy-2-naphthyl)2,2-dimethyl-pentanoic acid.

15. A composition as claimed in claim 1, wherein the compound B is 17α-ethinyl-17β-hydroxy-5(10-estren-3-one.

16. A composition as claimed in claim 1, wherein the compound B is m,m'-dimethyl-dihydrodiethylstilbestrol dipropionate.

17. A composition as claimed in claim 1, wherein the compound B is dimethisterone.

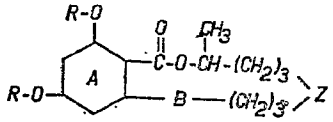
18. A composition as claimed in claim 1, wherein the compound B is norethindrone.

19. A composition as claimed in claim 1, wherein the compound B is chlormadinone acetate.

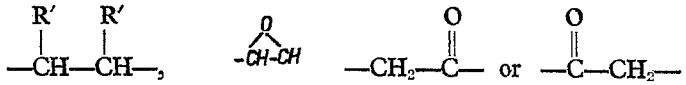
20. A composition as claimed in claim 1, wherein the compound B is medroxyprogesterone acetate.

21. A pharmaceutical or veterinary composition which comprises as active ingredients in admixture with an inert carrier,

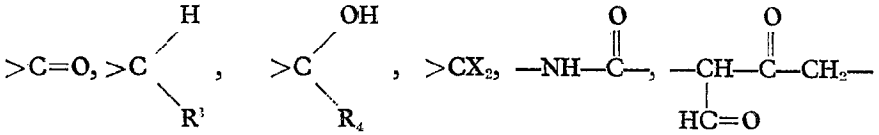
A. a compound having the structure:

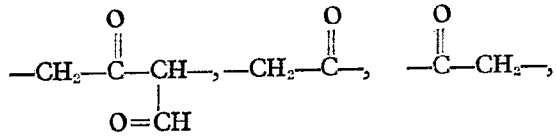
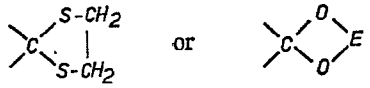


wherein R is a hydrogen atom, lower alkyl, lower saturated acyclic acyl or benzyl; B is $-CH=CH-$,



wherein R' is a hydrogen atom, fluoride, hydroxyl or $-N(R^2)_2$ wherein R^2 is a hydrogen atom, an alkyl having 1 to 15 carbon atoms, monocyclic aryl or an aralkyl having from 1 to 15 carbon atoms, Z is



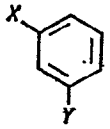
wherein R^3 is a hydrogen atom, an hydroxyl, a halogen atom, —OR^6 or —NHR^7, R^6 is lower alkyl, benzyl or lower saturated acyclic acyl and R^7 is a hydrogen atom, lower alkyl or monocyclic aryl; R^4 is alkyl, aryl, aralkyl, nitromethyl, aminomethyl, ethynyl, cyano or —R^9COOR^8 wherein R^8 is lower alkyl and R^9 is lower alkylene; X is a halogen atom, nitromethyl or aminomethyl; R^5 is lower alkyl, hydrogen atom, carbamyl or a monocyclic aryl; and E is an alkylene radical having 2 or 3 carbon atoms; with the proviso that when Z is

$>CH_2$, $>CHNHR^7$ or $>CHNHOH$,

B is $—CH_2—CH_2—$;

and

is a cyclo-hexane ring or a benzene ring of the formula

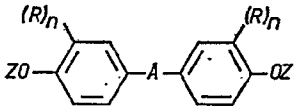


wherein X and Y are each a hydrogen, atom, nitro, amino, diazonium radical, cyano, hydroxyl, aryl, alkenyl, alkyl, acyl, alkoxy, carboxy, —SO_3H, —SO_3R^{10}, —SO_2Cl or —SO_2NHR^{11} wherein R^{10} is lower alkyl and R^{11} is a hydrogen atom, an alkyl having from 1 to 20 carbon atoms, monocyclic aryl or a halogen atom, with the proviso that when one of X and Y is carboxy the other of X and Y is a hydrogen atom, that R is lower alkyl or benzyl when X or Y is —SO_2Cl, and that when

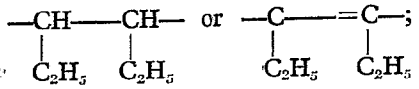
$>CHNHOH$, $>C=NOH$, $>C=NNHR^5$,

is a cyclohexane ring, B is $—CH_2—CH_2—$ and Z is $>CH_2$ or $>CHOR^6$.

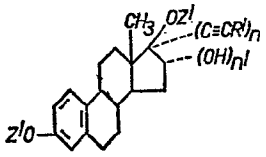
B. benzestrol; dienestrol; estrone; diethylstilbestrol or a derivative thereof as represented by the structure:



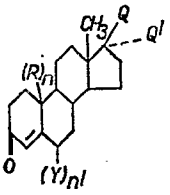
wherein A is



R is H or lower alkyl; Z is H, —PO_3, —SO_3, —R′ or —$\overset{O}{\overset{\|}{C}}R'$, where R′ is an aliphatic hydrocarbon radical having from 1 to 20 carbon atoms; n is 0 or 1 and when 0, R is H; estradiol or a derivative thereof as represented by the structure:

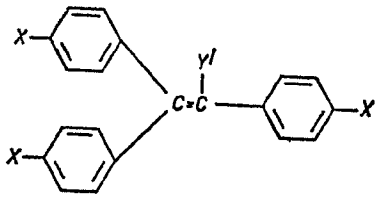


wherein Z′ is H, R or —$\overset{O}{\overset{\|}{C}}R$, where R is lower alkyl; R′ is H or lower alkyl; n and n′ are 0 or 1, and when 0, C≡CR′ is H; a progestogen having the structure:



wherein R is H or lower alkyl; Q is —OH or —$\overset{O}{\overset{\|}{C}}R$ and R is a lower alkyl; Q′ is H,

—C≡CH, —C≡C—R or —C(=O)R, wherein R is lower alkyl; Y is lower alkyl or a halogen atom and n and n′ are 0 or 1 and when n is 0, R is H and when n′ is 0, Y is H; or a tris-phenyl ethylene having the structure:



wherein X is OH, lower alkoxy or acyloxy and Y′ is a halogen atom or a hydrogen atom; the weight ratio of the active ingredient A to active ingredient B being from 300 to 1:1 to 300.

22. An animal feed composition comprising a composition claimed in any preceding claim and a nutrient diluent therefor.

23. A method of making an animal feed composition which comprises admixing a composition claimed in any of claims 1 to 21 with an edible nutrient carrier therefor.

24. A method of treating animals other than homo-sapiens for fertility control, estrogen therapy or to increase anabolic activity, which comprises administering to such animals a composition as claimed in any of claims 1 to 21 or approximately simultaneously but separately the active ingredients claimed in any of claims 1 to 21.

25. An animal feed composition when prepared by the method claimed in claim 23.

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